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14. ABSTRACT

The objective of this project was to elucidate the biological mechanisms and specific pathways that implicate the gaseous signaling molecule nitric oxide (NO) as a critical factor in producing the convulsions of central nervous system (CNS) oxygen (O2) toxicity and to obtain data that could be the basis for mathematical risk predictions of O2 convulsions. For the past 3 years, data obtained in this project support the hypothesis that NO· indeed contributes to CNS O2 toxicity by several mechanisms: a) by increasing the availability of NO· in the brain which in turn eliminates cerebral vasoconstriction, leading to hyperemia and the delivery of a toxic dose of oxygen; b) by stimulating NO· production and O2·- generation, both of which are implicated in the formation of ONOO-, a potent neurotoxic agent; c) by altering the excitatory/inhibitory balance in vulnerable brain regions during the early stage of extreme hyperoxia, prior to the appearance of O2 seizures. In rats protected with the inhibitor of NO production L-NAME no significant changes were observed in the excitotoxic index.

15. SUBJECT TERMS
High Pressure Oxygen Toxicity, Nitric Oxide, Seizures, CNS Oxygen Toxicity

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FINAL TECHNICAL REPORT

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PRINCIPAL INVESTIGATOR: Claude A. Piantadosi, MD

INSTITUTION: Duke Center for Hyperbaric Medicine and Environmental Physiology,

GRANT TITLE: Nitric Oxide and CNS O_2 Toxicity Biochemical Modeling and Risk Prediction.

AWARD PERIOD: 24 November 2000 - 31 January 2004

OBJECTIVE: To elucidate the biological mechanisms and specific pathways that implicate the gaseous signaling molecule nitric oxide (NO) as a critical factor in producing the convulsions of central nervous system (CNS) oxygen (O_2) toxicity. Although the precise mechanism by which CNS O_2 toxicity leads to convulsions is unknown, the fact that NO plays a critical role indicates that changes in its bioactivity (i.e. that fraction of its concentration that is available to exert a biological effect) lead to predictable responses *in vivo* that can be the basis for mathematical risk predictions of O_2 convulsions.

APPROACH: We measured *in vivo*, in anesthetized rodents, levels of reactive oxygen and nitrogen species (including NO), catacholamines, glutamate, and GABA as functions of PO_2 and time in the brain. We have correlated these with escape from cerebral vasoconstriction and with brain electrical activity. We postulated a biochemical mechanism of O_2 toxicity in order to derive a mathematical model to predict probability and time of onset of O_2 seizures. We also measured regional cerebral blood flow (rCBF) using hydrogen clearance, interstitial PO_2 and NO with microelectrodes, as well as significant neurotransmitters and products of brain metabolism, using microdialysis.

ACCOMPLISHMENTS: For the past 3 years, data obtained in our laboratory support our hypothesis that HBO decreases rCBF by increasing superoxide ($O_2^{\cdot-}$) production, which inactivates NO and produces vasoconstriction. This protects the brain against the damaging molecular effects of extreme hyperoxia. However, prolonged exposure to HBO in the 3 to 6 ATA range restores NO production and leads to generation of reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$), which is responsible for nitration of vascular and brain proteins, especially tyrosine and cysteine amino acid residues. Some (but not all) of these events interfere with molecular function. We also found critical roles for the depletion of the inhibitory amino acid GABA (gamma-aminobutyric acid) and the production of hydrogen peroxide and

ammonia by monoamine metabolism, which has allowed us to develop a biochemical model in which accelerated NO production leads to escape from vasoconstriction through the production of carbamyl phosphate.

NO-induced escape from autoregulation is followed by neuronal excitability, stimulation of metabolic activities that decrease seizure threshold and, ultimately, cause convulsions. Thus, we have been able to gather quantitative evidence in support of our hypothesis that changes in NO activity govern the escape of CBF from constrictor control that precedes neuronal excitotoxicity and predicts electrical hyperactivity during HBO exposure

CONCLUSIONS: $NO^{\cdot}$ indeed contributes to CNS O_2 toxicity by several mechanisms:

a) by increasing the availability of $NO^{\cdot}$ in the brain which in turn eliminates cerebral vasoconstriction, leading to hyperemia and the delivery of a toxic dose of oxygen;

b) by stimulating $NO^{\cdot}$ production and $O_2^{\cdot -}$ generation, both of which are implicated in the formation of $ONOO^-$, a potent neurotoxic agent. Rats pretreated with the systemic blocker of NO production L-NAME maintained a low CBF and did not show increases in interstitial $NO^{\cdot}$ and $ONOO^-$ or EEG signs of oxygen toxicity;

c) by altering the excitatory/inhibitory balance in vulnerable brain regions during the early stage of extreme hyperoxia, prior to the appearance of O_2 seizures. In rats protected with L-NAME no significant changes were observed in the excitotoxic index.

SIGNIFICANCE: These data provide the first direct correlation between increased NO production and the onset of hyperoxic vasodilation in prolonged HBO_2 exposure.

Our biological data provide essential parameters and mechanistic interrelationships needed to construct a basic biochemical model to describe, predict, and ultimately, perhaps, to delay the early events of O_2 toxicity.

PATENT INFORMATION: N/A

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